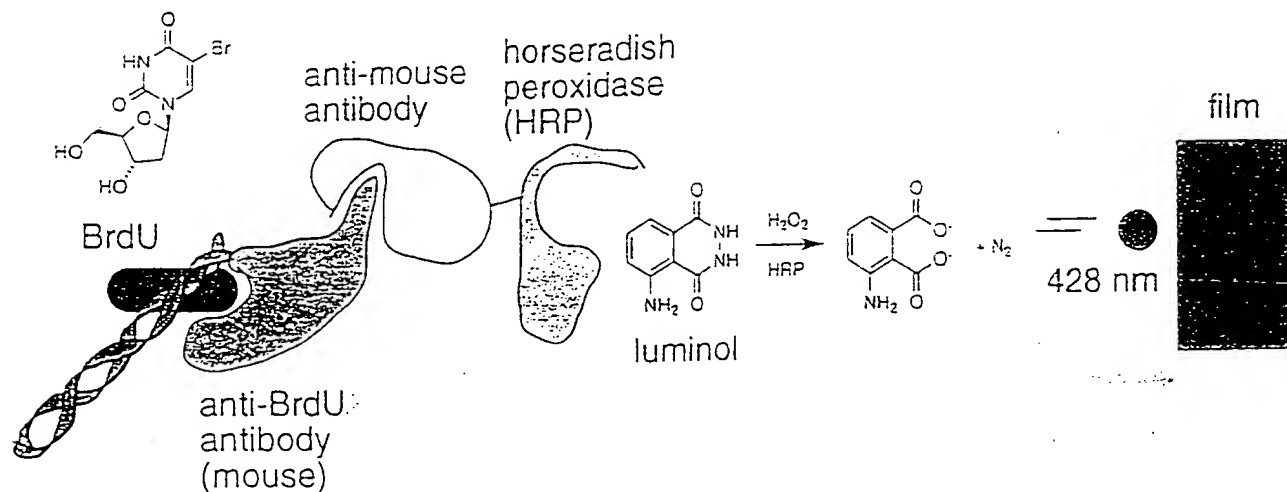
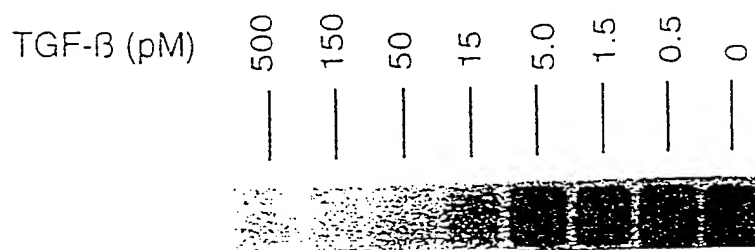


An immunoassay for detecting DNA synthesis in high density arrays of mammalian cells

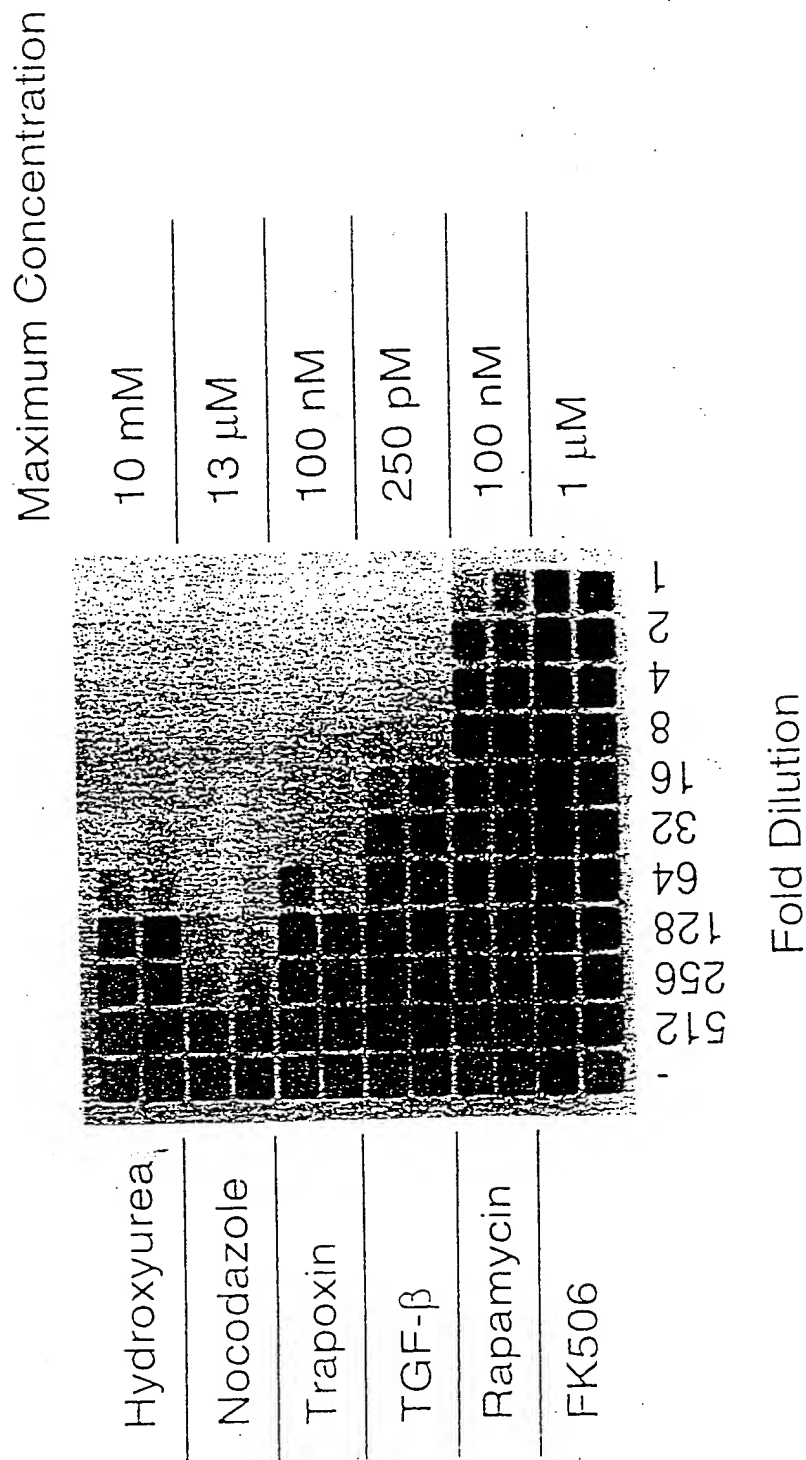
(a)



(b)



Numerous Antiproliferative Agents Inhibit BrdU Incorporation

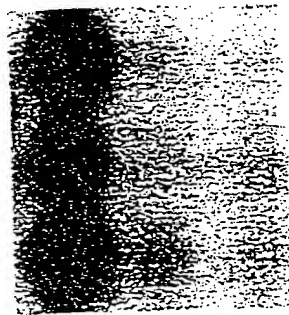


BrdU cyto blot on 2000 Mv1Lu cells, 43 hour treatment + 7 hours with BrdU

BrdU Incorporation Can be Efficiently Detected in 1536 Well Plates in 2 μ L Droplets

Treatment
BrdU TGF- β

+	-
+	+
-	-

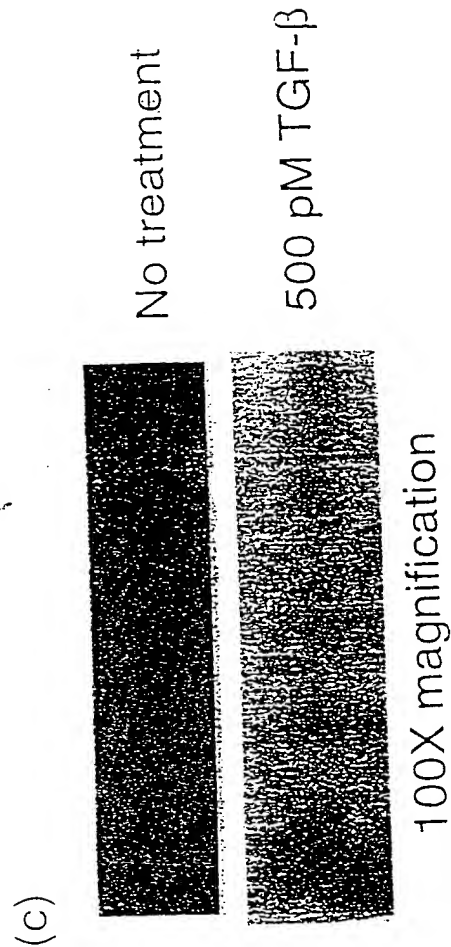
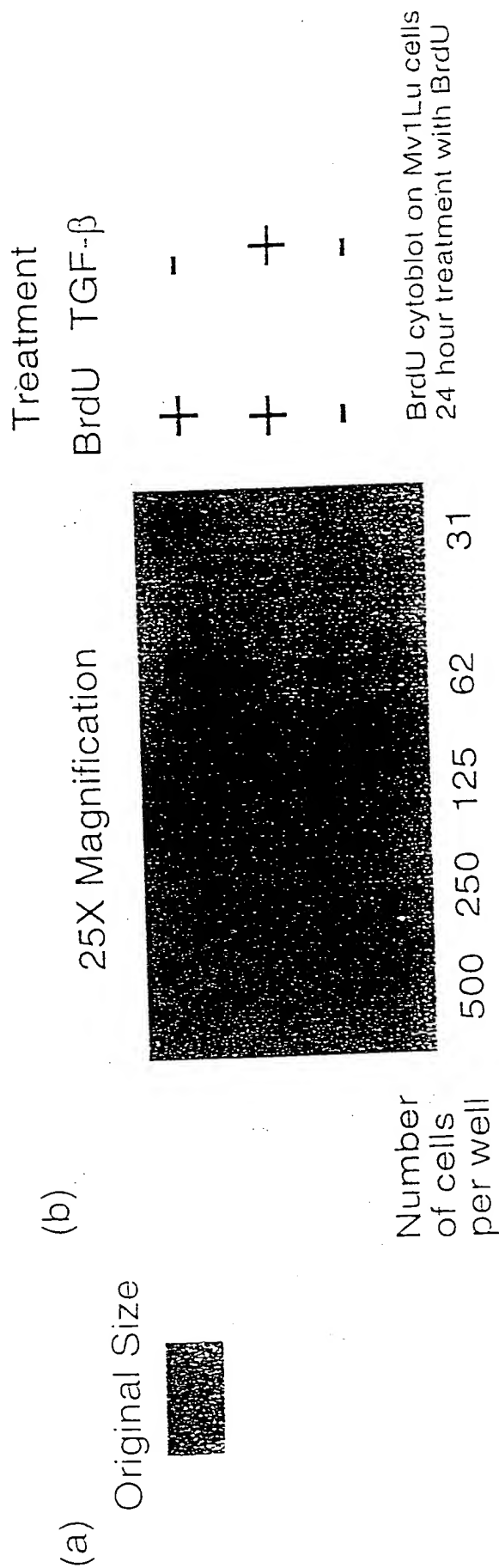


Actual Size

36X Magnification

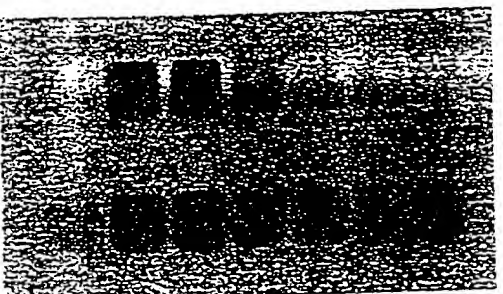
BrdU cytotblot on 500 Mv1Lu cells, 28 hour treatment + 7 hours with BrdU

BrdU incorporation is detected in 6144 well plates with 62 cells in 250 nL volumes



4
7.4

Hyperacetylation of histone H4 is detected with a cyto blot



No treatment, no primary Ab

No treatment

0.5% serum

80 pM TGF- β

300 nM trichostatin A

100 nM trapoxin

250 nM nocodazole

anti-acetylated H4 blot

4X magnification

4000 A549 cells

00361576-072799

Fig 5

Phosphonucleolin is detected with the antibody TG-3 in a cyto blot

TG-3 blot



No treatment



500 nM nocodazole
24 hour treatment

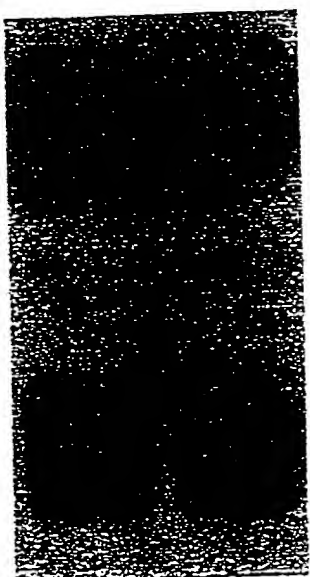
500 1000 2000 4000

Number of cells (A549)

4X magnification

Detecting phosphorylation of histone H3 as a marker of mitosis

100 nM
nocodazole No treatment



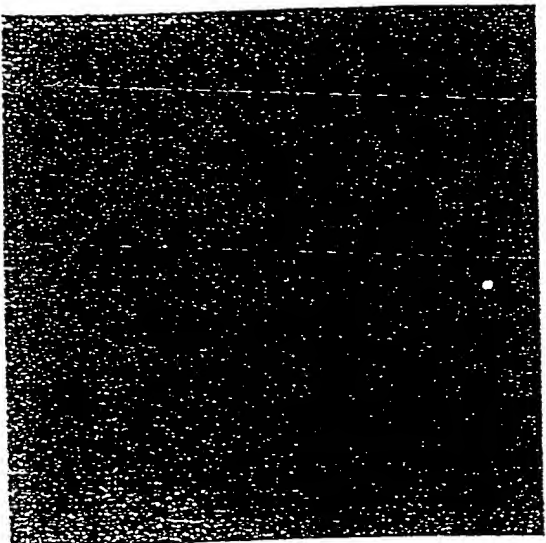
16 hour treatment, anti-phospho histone H3 mitosis marker

4000 A549 cells

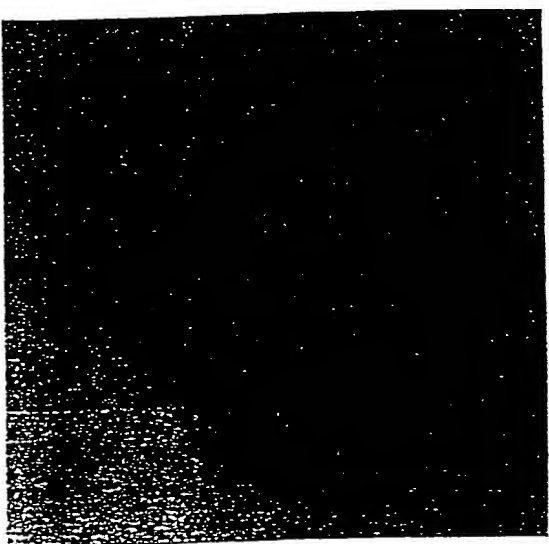
00361575-072799

6h
Fry

Detection of phosphonucleolin with TG-3 in 1536 well plates with a cyto blot



No treatment



250 nM nocodazole



size of one well

4X magnification
TG-3 blot
300,000 HeLa cells / mL

09361576-073399

Fig 28 bc

00220-9457960

1

anti-acetylated H4 antibody

HaCaT

A549



NT no 1° Ab

NT

0.5% serum

80 pM TGF- β

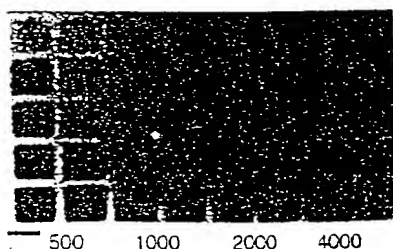
300 nM TSA

100 nM trap

250 nM ncdz

2

anti-acetylated H4 antibody



NT

4 hr

8 hr

12 hr

24 hr

trapoxin treatment

500 1000 2000 4000

Number of cells

3

anti-phospho histone H3
antibody (mitotic marker)



NT

4 hr

8 hr

12 hr

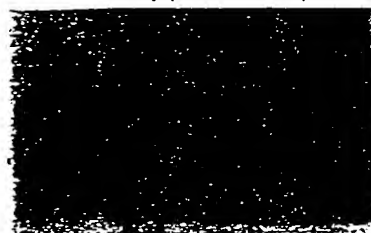
24 hr

ncdz treatment

500 1000 2000 4000
Number of cells

4

anti-phosphonucleolin
antibody (mitotic marker)



NT

4 hr

8 hr

12 hr

24 hr

ncdz treatment

500 1000 2000 4000
Number of cells

5

anti-phosphonucleolin
antibody (mitotic marker)



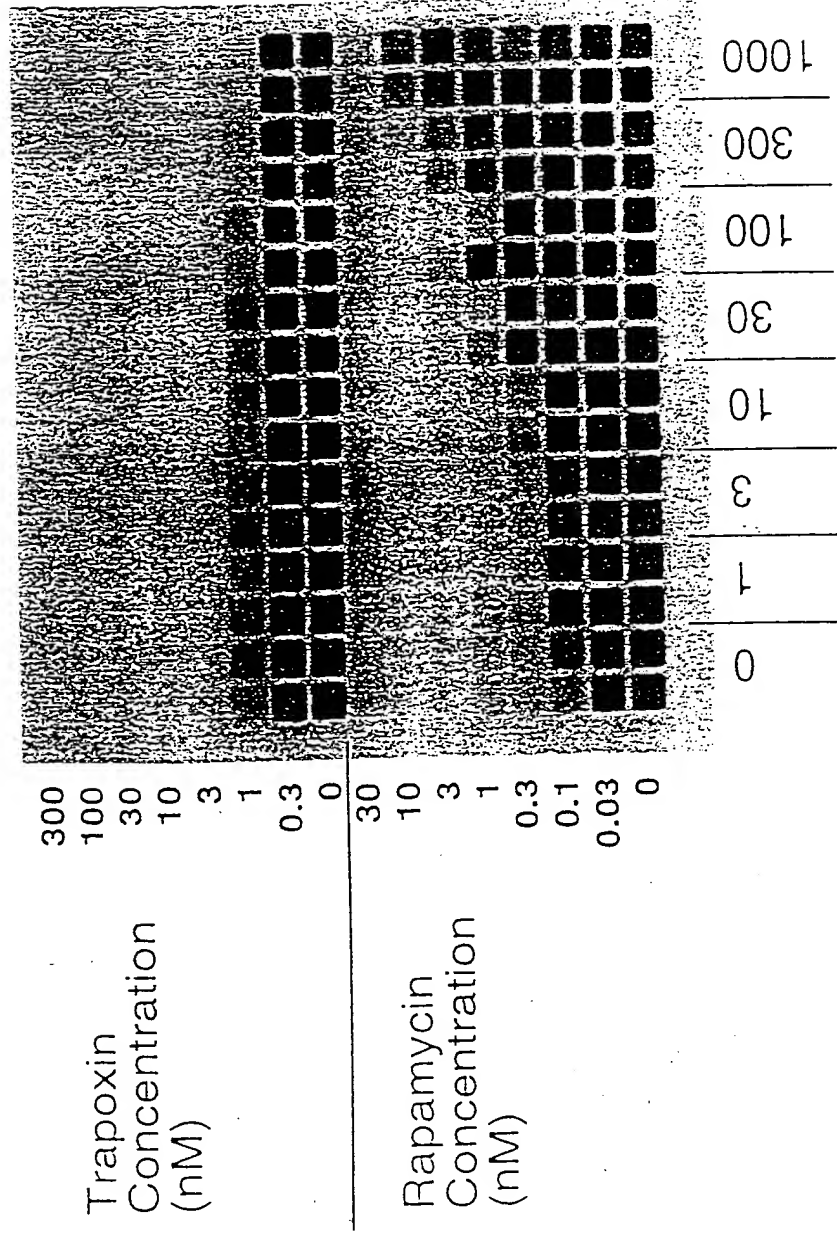
250 nM ncdz

NT

6144 well plate

Fig 6d

FK506 Suppresses the Antiproliferative Effect of Rapamycin but not Trapoxin



FK506 Concentration (nM)

BrdU cyto blot on 2000 6F cells, 44 hour treatment + 7 hours BrdU treatment

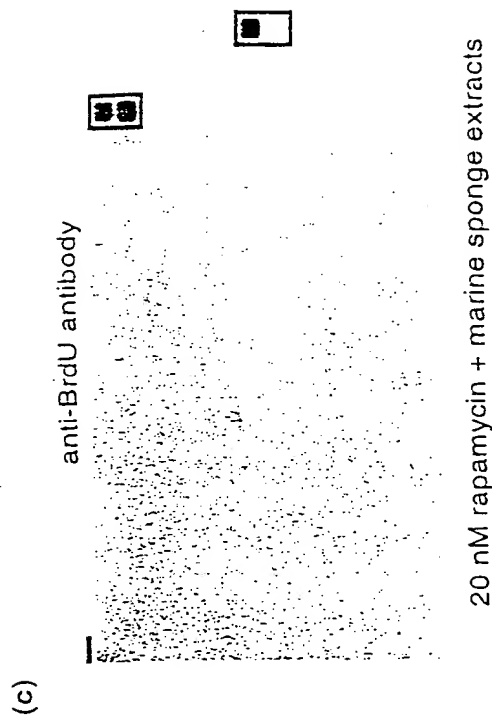
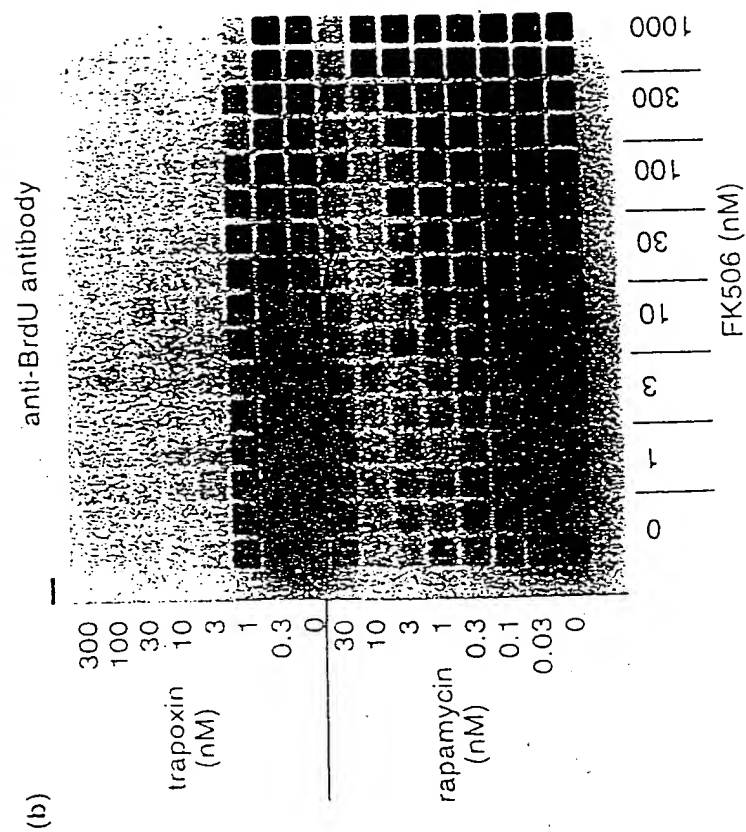
[illegible]

Fig. 9.

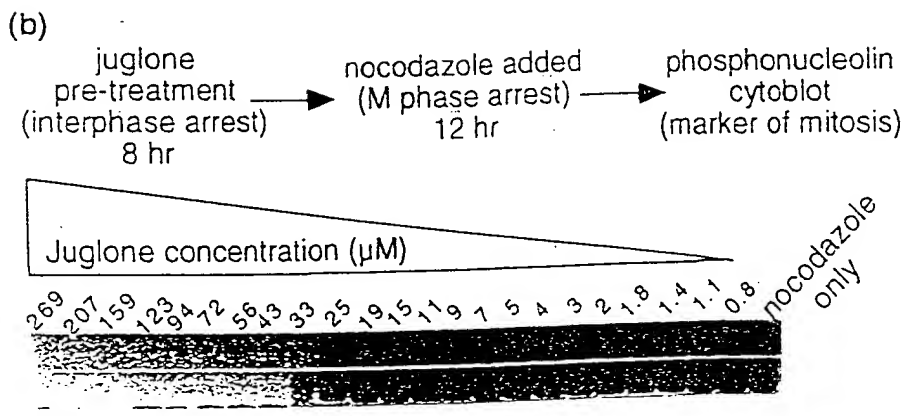
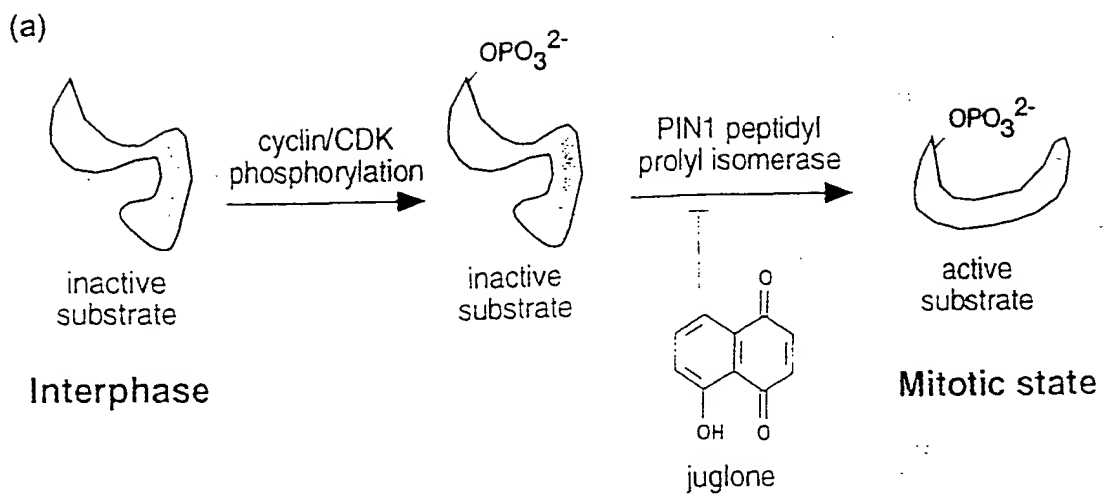
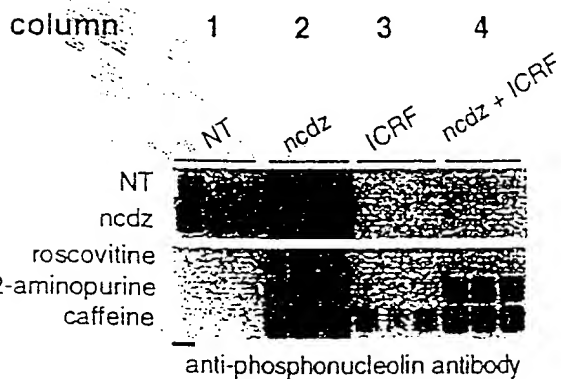
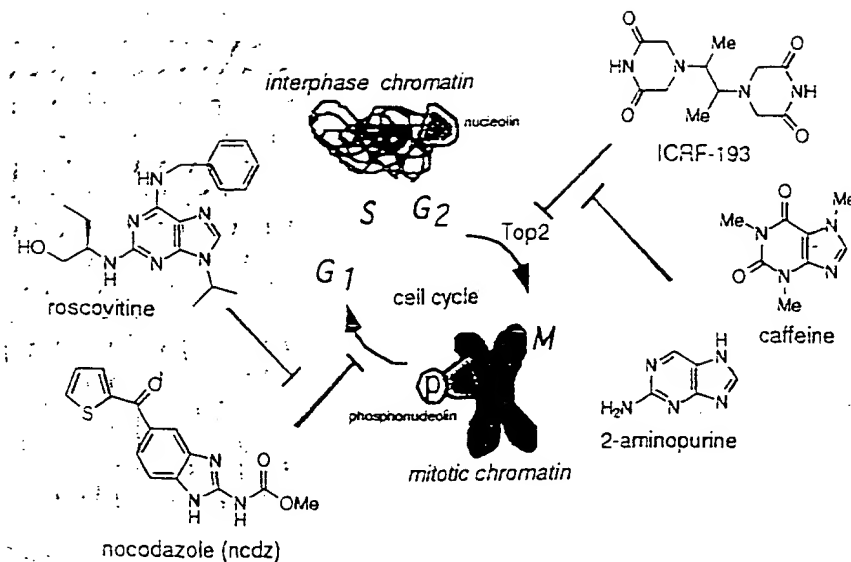


Fig 10

(a)



(c)

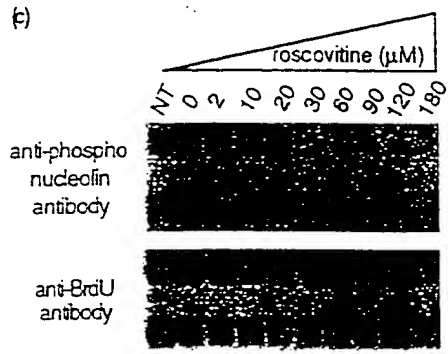
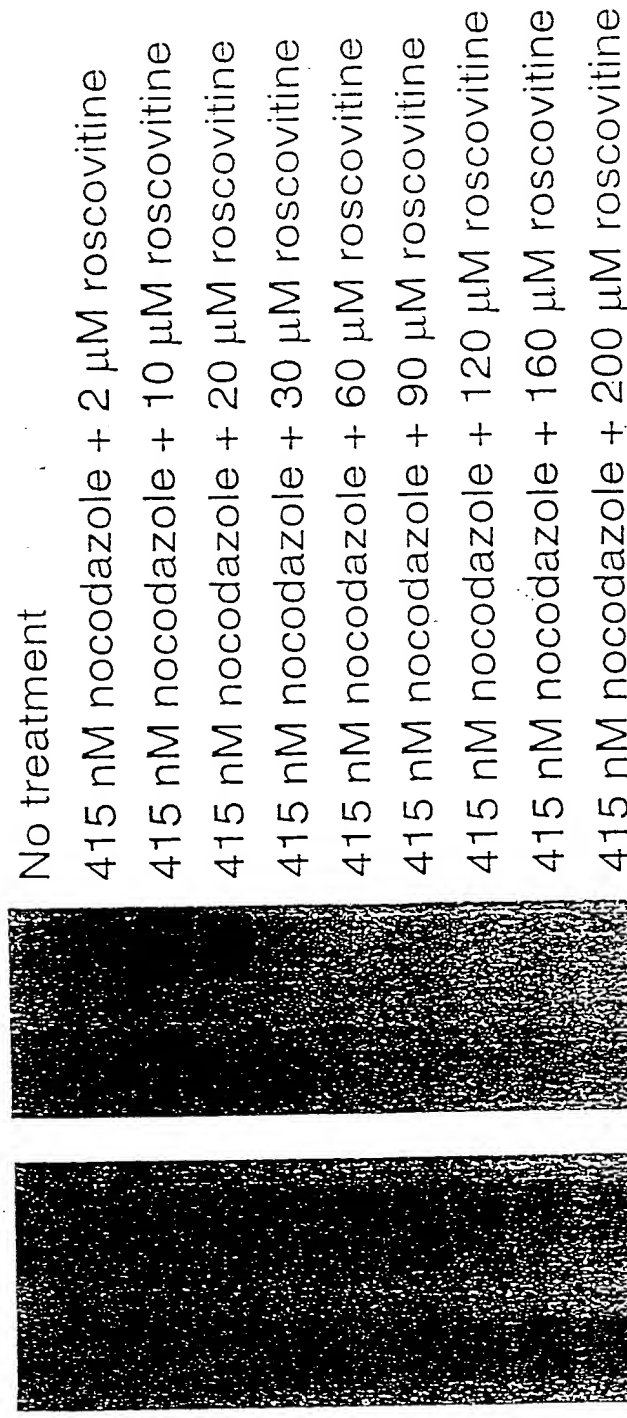


Fig 11

Nocodazole prevents BrdU incorporation and induces the accumulation of phosphonucleolin

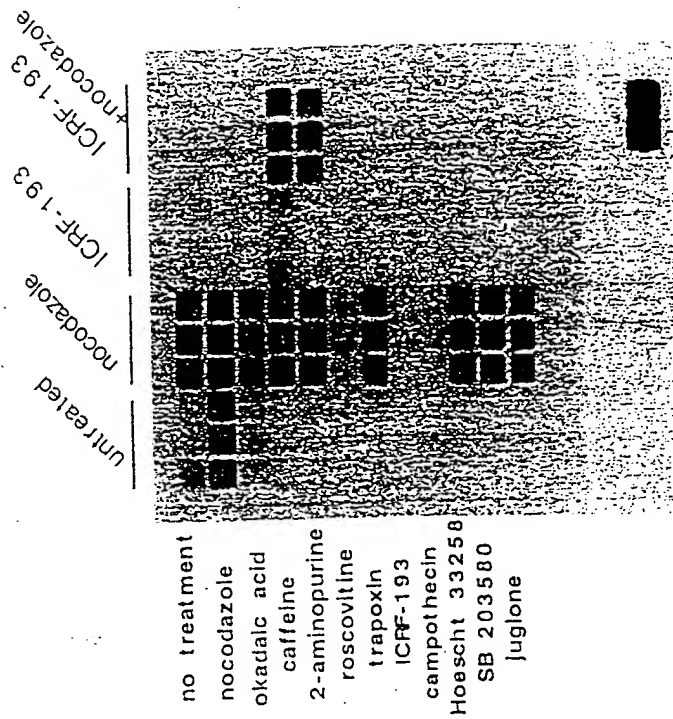
Roscovitine suppresses only the latter effect



anti-BrdU TG-3

4000 HeLa cells
4X magnification

Suppression of a DNA damage-independent topoisomerase inhibitor-induced G2-checkpoint arrest by caffeine and 2-aminopurine



TG-3 blot

18 hr treatment

A549 cells 384 well plate
~6000 cells/well

Fig 11e

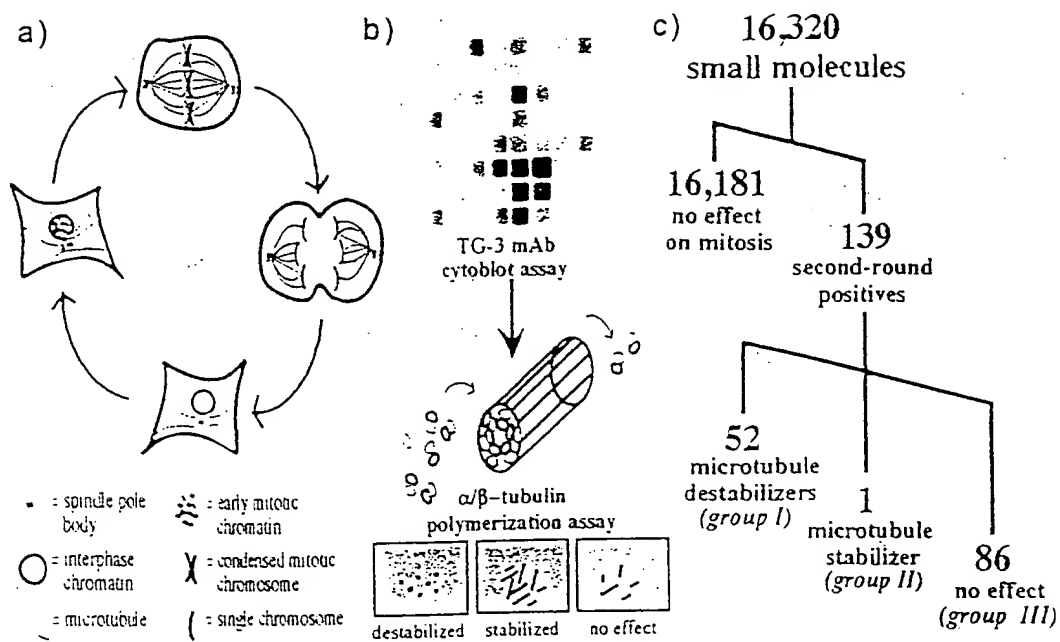
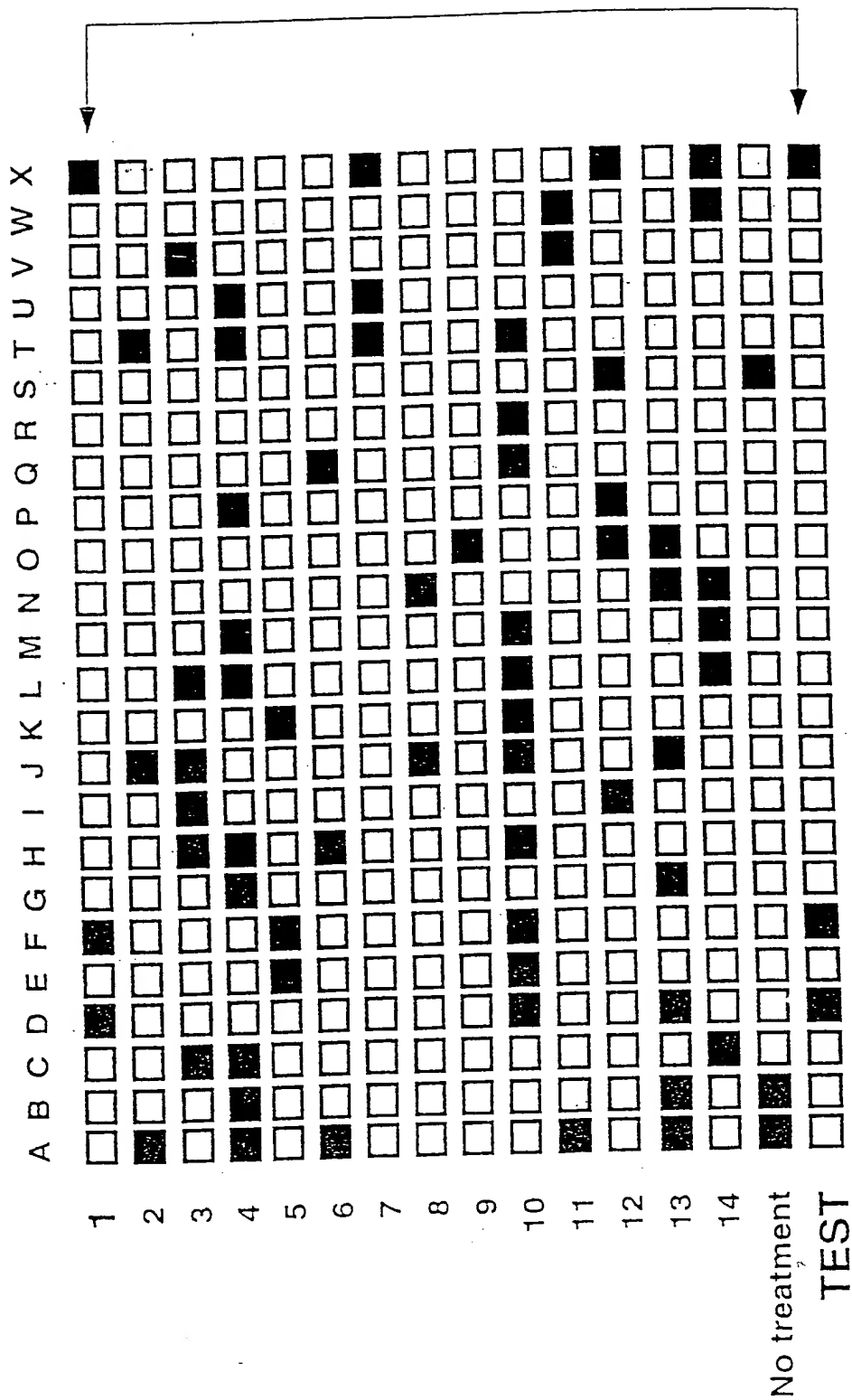


Figure 1. Screening for small molecules that affect the mammalian cell division cycle. a) Schematic of cell cycle events involved in mitotic chromosome segregation. b) summary of screening steps. c) division of small molecules into three groups based on their effects on the stability of purified microtubules.

Fig. 12

Functional Fingerprinting of a Test Compound with 24 Different Antibodies



000009090909

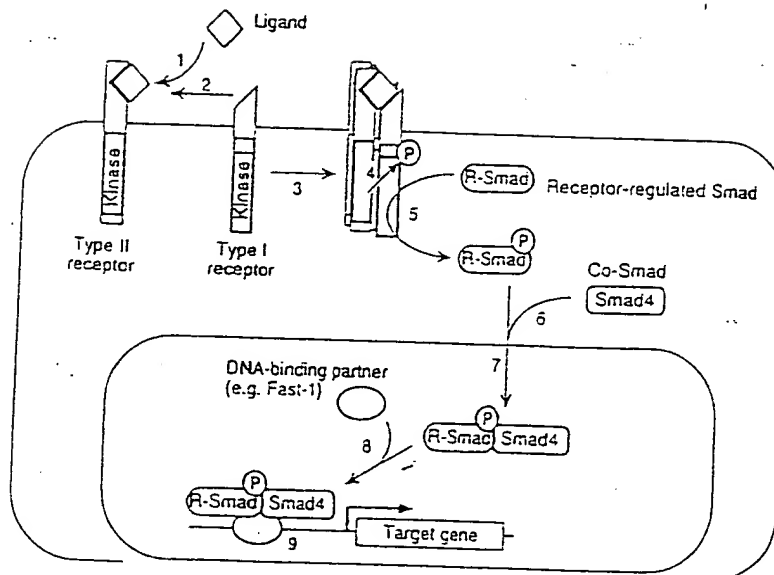


FIGURE 14

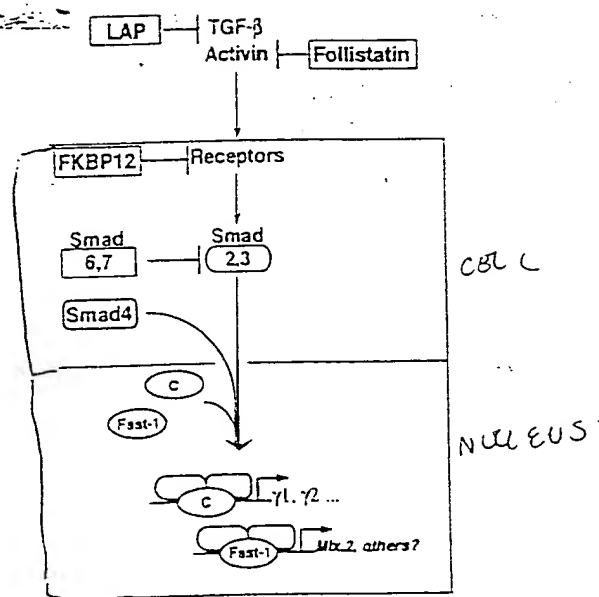


FIGURE ~~14~~
15

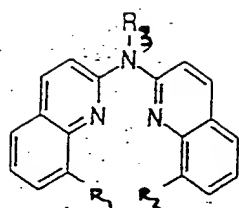


FIGURE ~~2~~
16

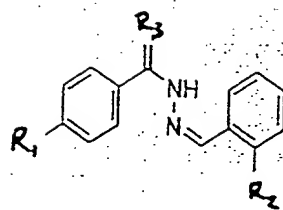
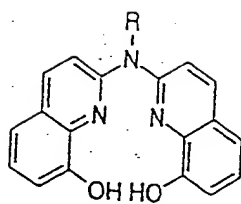
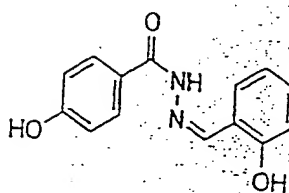


FIGURE ~~7A~~
17

FIGURE 18



1a R = Me
1b R = H
1c R = nBu



2

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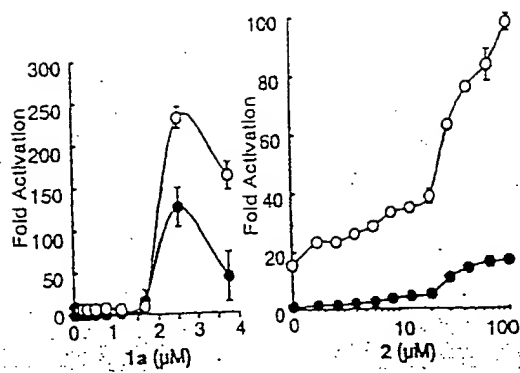


Figure ~~18~~
19

Fig. 20

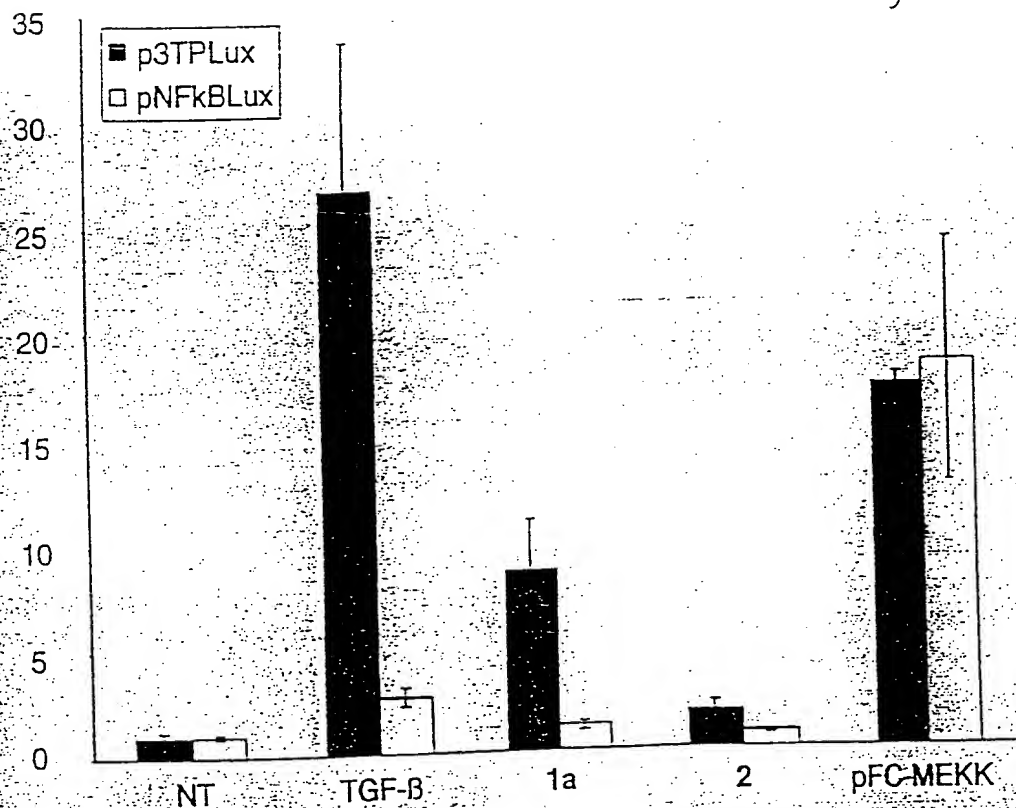


Figure 22. Reporter gene specificity of 1a and 2. 1×10^5 Mv1Lu mink lung epithelial cells were transiently transfected with 400 ng reporter gene (p3TPLux or pNFkBLux) alone or with 50 ng pFC-MEKK using DEAE dextran [ref] in 12-well dishes and subsequently cultured in mink medium with 10% FBS for 23 hours. The cells were then treated with nothing (NT), 400 pM TGF- β 1 (TGF-B), 2.5 μ M 1a, or 50 μ M 2 in mink medium with 0.2% FBS. After 24 hours, luciferase activity was measured as described previously [ref].

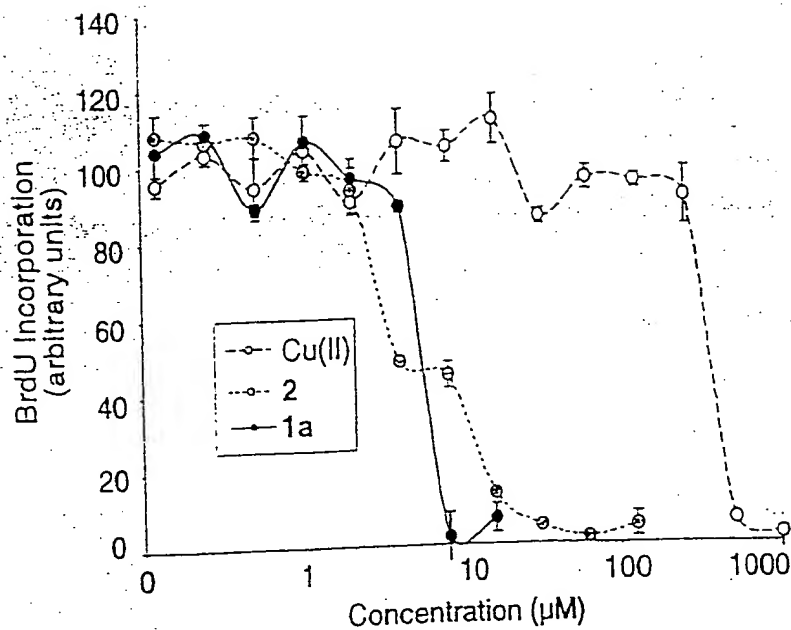


Figure 81: 1a, 2 and Cu(II) inhibit BrdU incorporation in mink lung epithelial cells. 2000 cells were seeded in each well of a 384 well plate in the presence of the indicated concentrations of reagents. The BrdU cyto blot assay was performed as described previously [ref].

Fig. 21

Fig. 22

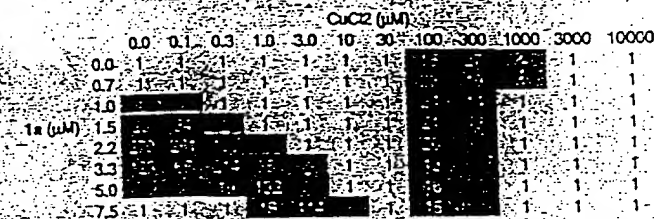
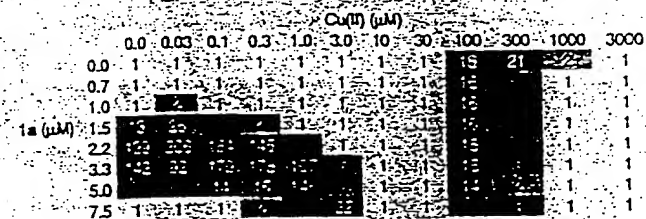
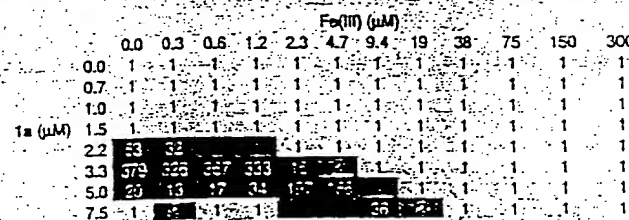
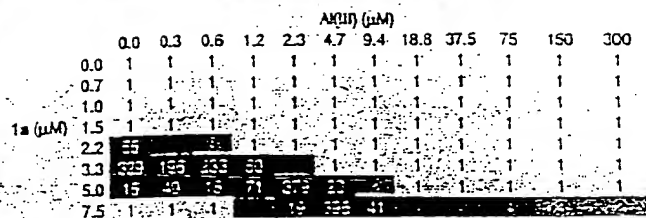
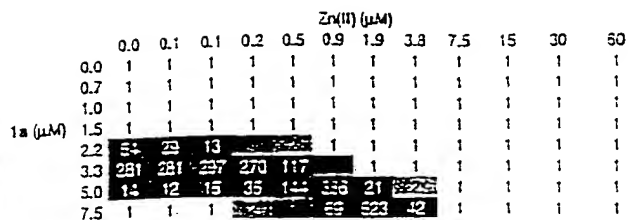
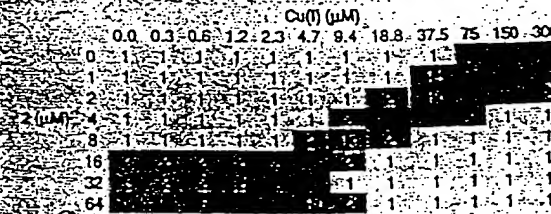
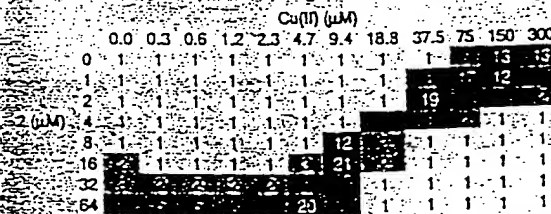
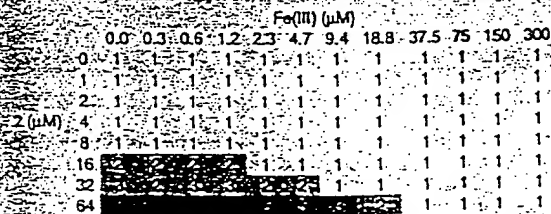
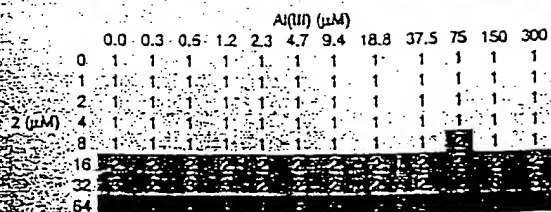
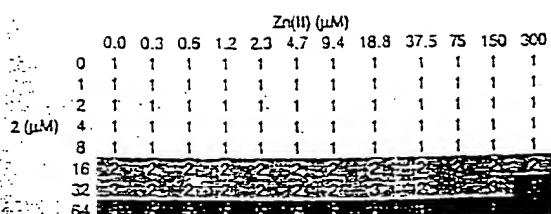


Fig. 22. Effect of metal ions on the activity of 1a and 2. Two-dimensional dose-responses are shown of metal against compound. The interior boxes indicate fold activation and are colored according to the following greyscale: 0-1.5 = white, 1.5-3 = light grey, 3-5 = grey, 5-12 = dark grey, >12 = black. All experiments were performed in quadruplicate.

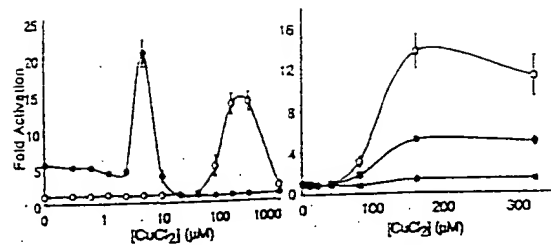


Figure ~~22~~
23

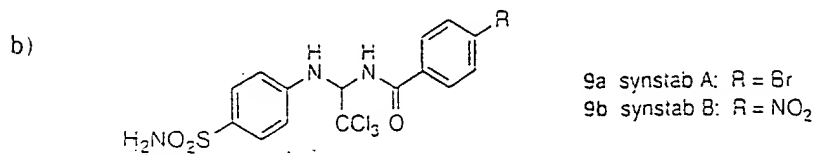
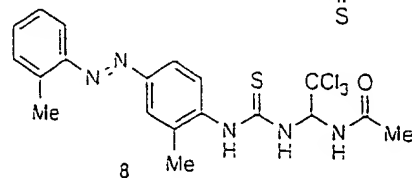
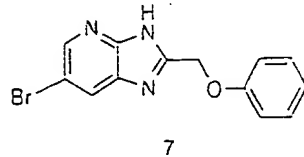
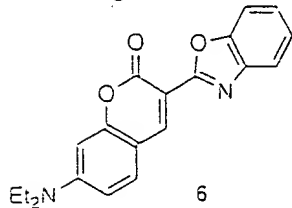
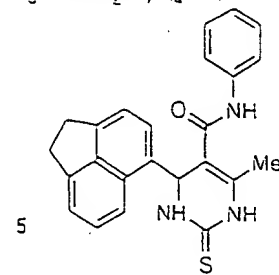
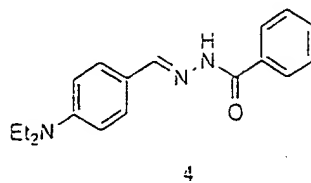
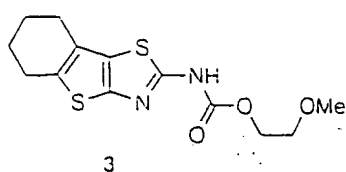
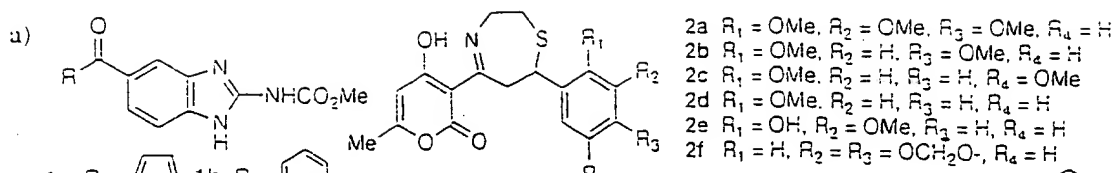


Fig. ~~23~~ 24

Cc1cc(O)c2c(c1)oc(=O)c2C3=NC4CCSC4=N3c5cc(R1)cc(R2)cc5R3

- 2g $R_1 = H, R_2 = OMe, R_3 = OMe, R_4 = OMe$
 2h $R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = OMe$
 2i $R_1 = OMe, R_2 = OMe, R_3 = H, R_4 = H$
 2j $R_1 = H, R_2 = H, R_3 = OMe, R_4 = H$
 2k $R_1 = H, R_2 = OMe, R_3 = OH, R_4 = H$
 2l $R_1 = H, R_2 = OH, R_3 = H, R_4 = H$
 2m $R_1 = OH, R_2 = Cl, R_3 = H, R_4 = Cl$
 2n $R_1 = H, R_2 = H, R_3 = Cl, R_4 = H$
 2o $R_1 = H, R_2 = Br, R_3 = H, R_4 = H$
 2p $R_1 = H, R_2 = H, R_3 = \text{isopropyl}, R_4 = H$
 2q $R_1 = H, R_2 = H, R_3 = Me, R_4 = H$

Fig. ~~24~~ 25

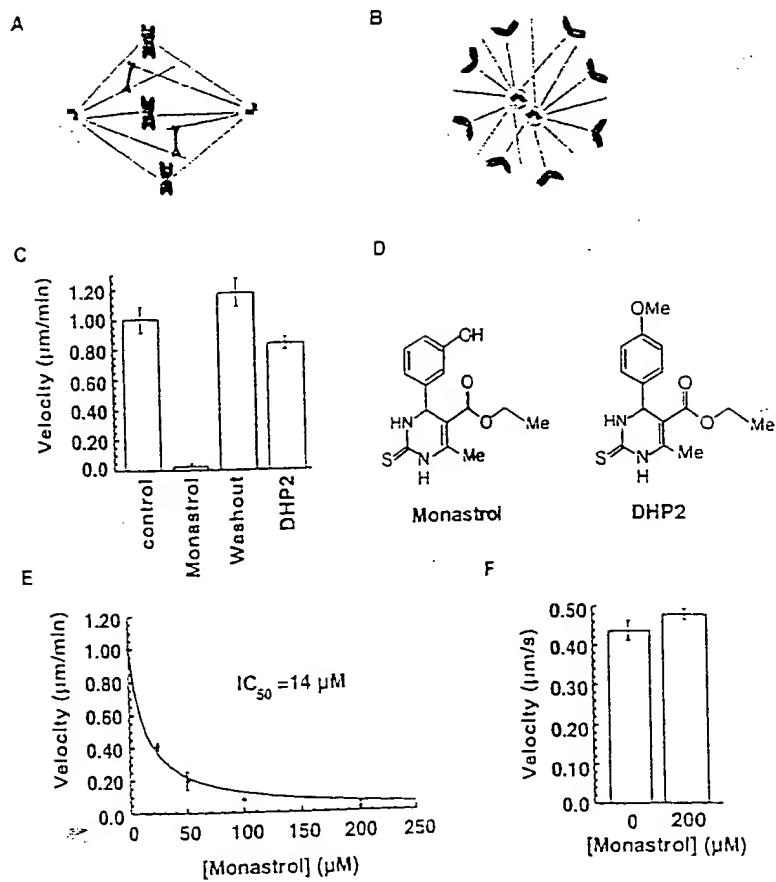
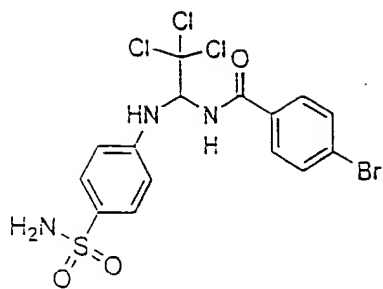
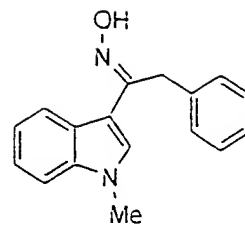


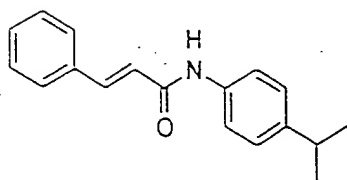
Fig. 25 26



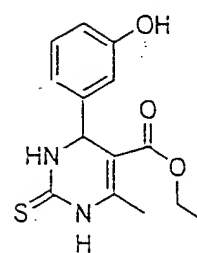
A



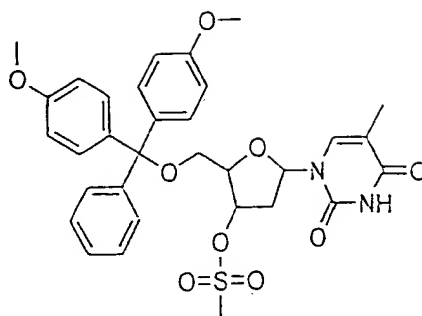
B



C



D



E

Figure ~~26~~ 27